Shoji SHIBATA*, Osamu TANAKA*, Ushio SANKAWA*, Yukio OGIHARA*, Rumiko TAKAHASHI*, Shujiro SEO*, Dun-Mei YANG* and Yumiko IIDA*: **The constituents of *Acroscyphus sphaerophoroides* Lév.**

柴田承二*・田中 治*・三川 潮*・荻原幸夫*・高橋ルミ子*・妹尾修次郎*・楊 敦美*・飯田由美子*: カニメゴケの成分

Acroscyphus sphaerophoroides Lév. is a morphologically very characteristic lichen of the Sphaerophoraceae in Coniocarpineae occurring as one species in one genus. It has been recorded that this lichen grows in some district of higher altitude in Asia and South America of the Circum-Pacific Area. In Japan it has been found at Mt. Daisetsu (Hokkaido), Mt. Ontake (Kiso), Mt. Mitsudake (Japan Alps) and Mt. Kimpu (Chichibu) growing to form small colonies[1]. Recently large colonies of this lichen were collected in Himalaya area by the Botanical Expedition to Eastern Himalaya organized by the University of Tokyo**: At Jongin (400 m height) in Sikkim***, at Singke-La (5000 m height) and Yale-La (4600 m height) in Bhutan****.

By the preliminary micro-scale experiment, we confirmed already that this lichen of both Japanese and Himalayan origins contains the same chemical constituents. Using a fairly sufficient amount of material collected in Bhutan, several constituents of this lichen have been isolated in crystalline forms, whose identifications have been performed.

The lichen thalli were extracted with ether or petroleum ether, and the extracts were fractionated using chromatography as shown in Fig. 1 to obtain the compounds tentatively called B, B′, C, E, F, G, G′ and H, respectively.

For the detection of the constituents, the extracts of lichen were at first separated roughly by the preparative thin layer chromatography on a silica gel H plate using benzene-acetone (9 : 1) being developed twice, and the separated bands (1), (2) and (3) were chromatographed again using benzene-hexane (3 : 2), benzene-acetone (20 : 1), and benzene-acetone (9 : 1), respectively. The thin layer chromatograms are shown in Fig. 2.

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*** Collected by Mr. M. Togashi.
**** Collected by Dr. O. Tanaka.

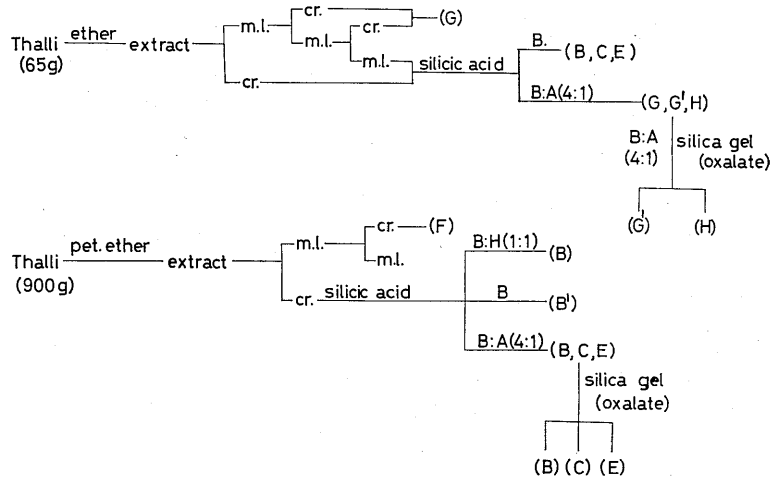


Fig. 1. Separation process of the constituents of *Acroscyphus spaerophoroides*.

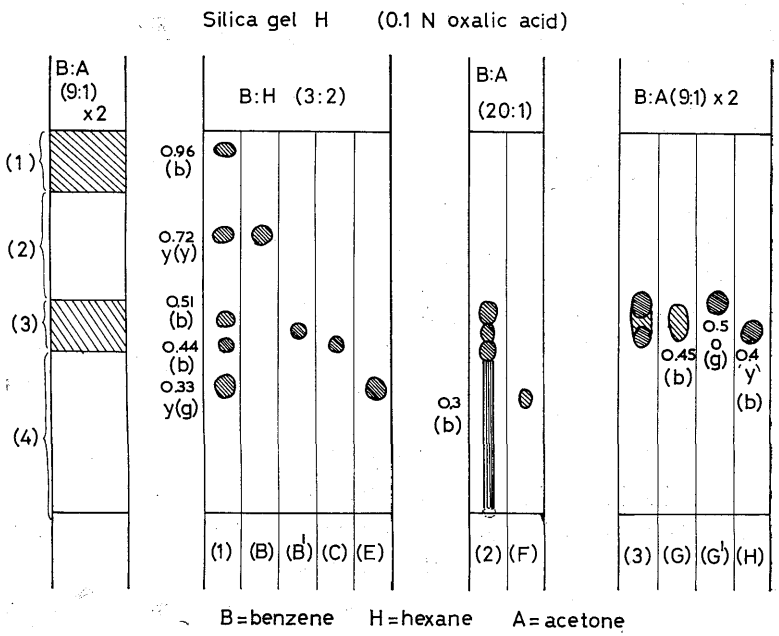


Fig. 2. Thin layer chromatograms of the constituents of *Acroscyphus sphaerophoroides*.

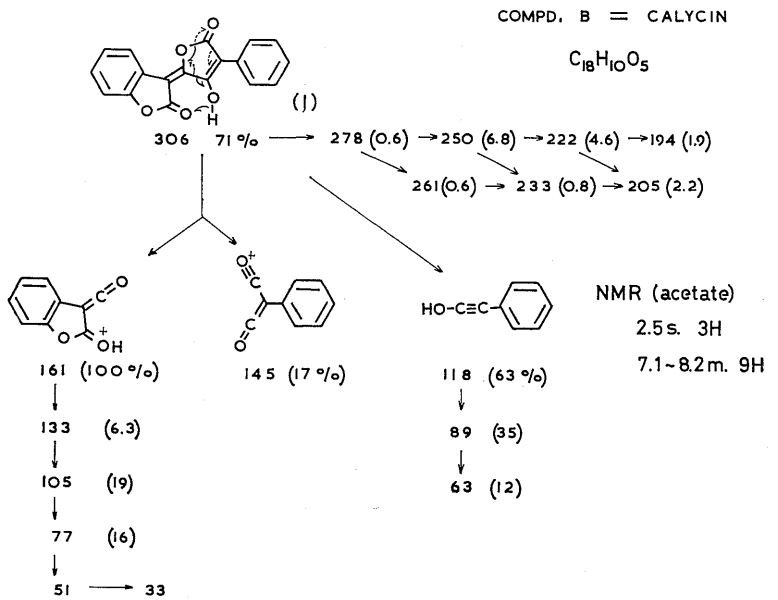
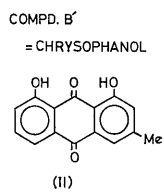


Fig. 3. The fragmentation pattern of mass spectrum of calycin.

The identifications of the chemical constituents were made mainly by mass and NMR spectral methods in a micro-scale experiment due to the limited amount of the material.

Compound B (=Calycin (I))[2,3], orange crystals, m. p. 237–240°, yield: 300 mg from 900 g. thalli—The monoacetate of this compound, m. p. 179°, showed in the NMR spectrum (in $CDCl_3$) a signal of $OCOCH_3$ at δ 8.47 (3H, s) and complex signals at δ 7.1–8.2 (aromatic protons (9H)). The mass spectrum of the compound B showed the molecular ion peak at 306 (Calcd. for $C_{18}H_{10}O_5$: 306) and the fragmentation pattern suggested that it is a pulvic acid derivative having 5 oxygen atoms. The mixed fusion and the comparison of IR spectrum ($IR_{max}^{CHCl_3}$ cm^{-1}: 1800, 1630, 1027, 890) proved the identity of the compound B with the authentic sample of calycin (I).

Compound B′ (=Chrysophanol (II))[4] orange crystals, m. p. 191–193°, yield: 10 mg from 900 g thalli—The positive magnesium acetate reaction (red: α-hydroxyanthraquinone derivative), and the Rf-value of thin layer chromatogram suggested that the compound B′ would



be chrysophanol (II). The identification of B′ with chrysophanol was made by a mixed fusion and a comparison of IR spectra.

Compound C (=Atranorin (III))[2]—colourless crystals, m.p. 194–196°, yield: 250 mg from 900 g thalli—The mass spectrum gave a weak M^+ peak at 374 (Calcd. for $C_{19}H_{18}O_8$: 374) and no remarkable peak down to m/e 196. Strong peaks were observed at 179, 164, 150, 136. Such a fragmentation pattern of the mass spectrum suggested a depside structure. The appearance

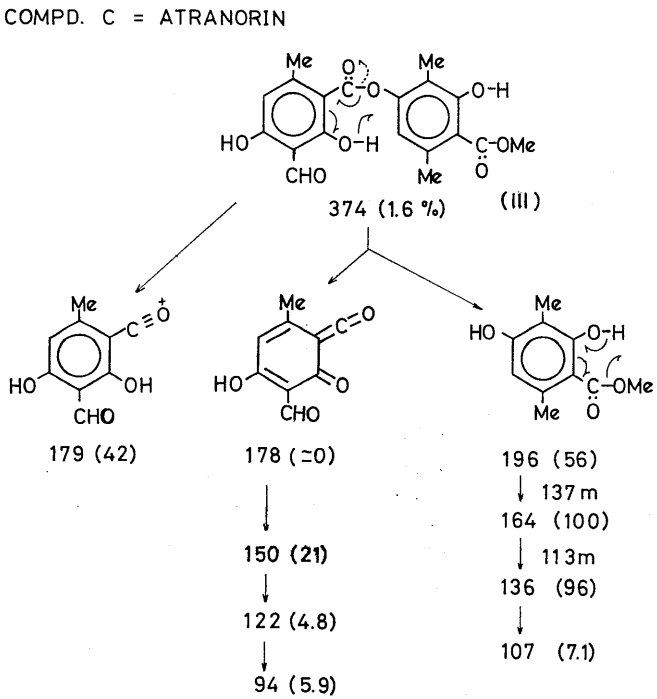


Fig. 4. The fragmentation pattern of mass spectrum of atranorin.

of peaks at 196 ($C_{10}H_{12}O_4$) and 164 (:196-32) showed a loss of MeOH to suggest the presence of a methyl ester grouping in the molecule of Compound C, whose identity with atranorin (III) was established by a mixed fusion and a comparison of IR spectra.

Compound E (=Usnic acid (IV))[2], yellow crystals, m.p. 198–201°, yield: 400 mg from 900 g thalli—It gave a positive Ehrlich reaction with *p*-dimethylaminobenzaldehyde and HCl. The colour reaction and the mass spectrum (M^+:

344 (Calcd. for $C_{18}H_{16}O_7$, 344), strong peaks at 260 (M-84), 232 (M-111), 43 (CH_3CO); weak peaks at 329 (M-15), 301 (M-43)) suggested the identity of the compound E with usnic acid, which was established by the comparison of IR spectra with the authentic specimen. The melting point (m. p. 198-201°) and $[\alpha]_D^{20}+380°$ (c: 0.08-0.13) (in $CHCl_3$) of the compound E observed after twice recrystallization from benzene are somewhat lower than those data (m. p. 205-207°, $[\alpha]_D^{20}+490°$ (c: 0.08-0.15) of (+) usnic acid isolated from *Cladonia mitis* and purified by the same manner. The compound E recrystallized five times gave m. p. 201-204°, and $[\alpha]_D^{20}+410°$, which is regarded to be a quasi-racemate of usnic acid consisting of 90% (+) form and 10% (−) form.

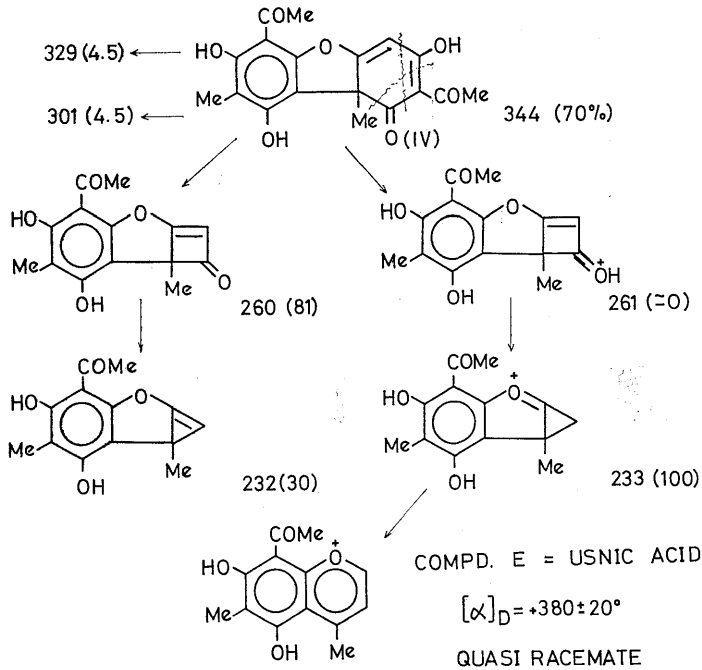


Fig. 5. The fragmentation pattern of mass spectrum of usnic acid.

Compound F (=Zeorin (V))[2] colourless crystals, m. p. 212°, yield: 70 mg from 900 g of thalli—The mass spectrum of compound F showed M^+ at 444 (Calcd. for $C_{30}H_{52}O_2$: 444) and the fragment peaks at m/e 426, 383, 357, 207 and 189. The identity of the compound F and zeorin was established by a

mixed fusion and a comparison of IR spectra with the authentic specimen.

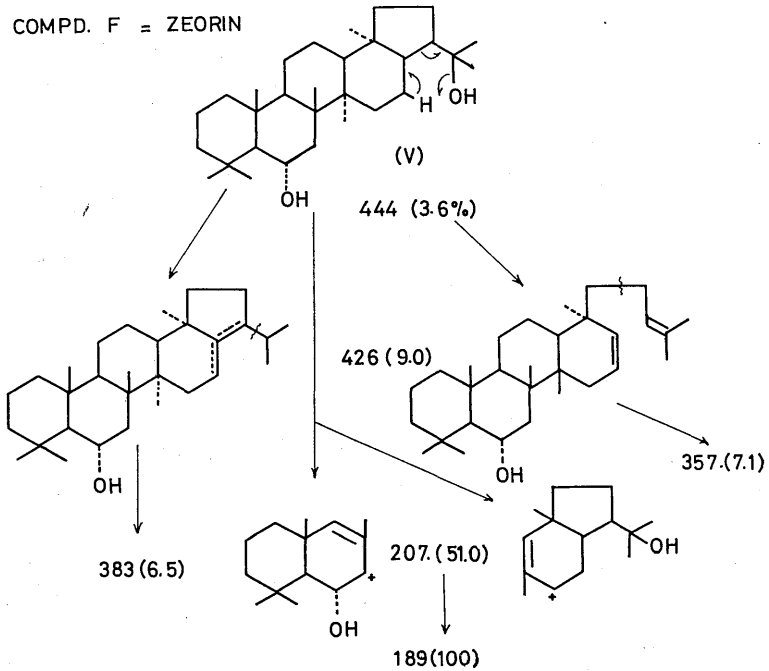


Fig. 6. The fragmentation pattern of mass spectrum of zeorin.

Compound G (=Gyrophoric acid (VI))[2], colourless crystals, m. p. 250°, yield: <10 mg from 65 g thalli—The colour reaction with bleaching powder (red), the NMR spectral pattern (in d_6-DMSO): δ 2.45, 6.30, 6.70 (s 9:2:4) of this compound and its acetate, m. p. 230° (12H:4 $COCH_3$) and the mass spectrum giving the same fragmentation pattern with that of lecanoric acid (m/e 318, 168, 150, 122, 94) and additional very weak peaks in the higher region 318) suggested that the compound G would be gyrophoric acid. The identity was established by the comparison of the IR spectra with the authentic sample.

Compound G' (=(+) Skyrin (VII))[5,6,7], orange crystals, m. p. >250°, yield: 10 mg from 65 g of thalli—The colour reaction with magnesium acetate (red), conc. H_2SO_4 (red to olive green) and the Rf-value of thin layer chromatogram suggested the identity of this compound with skyrin, which has been

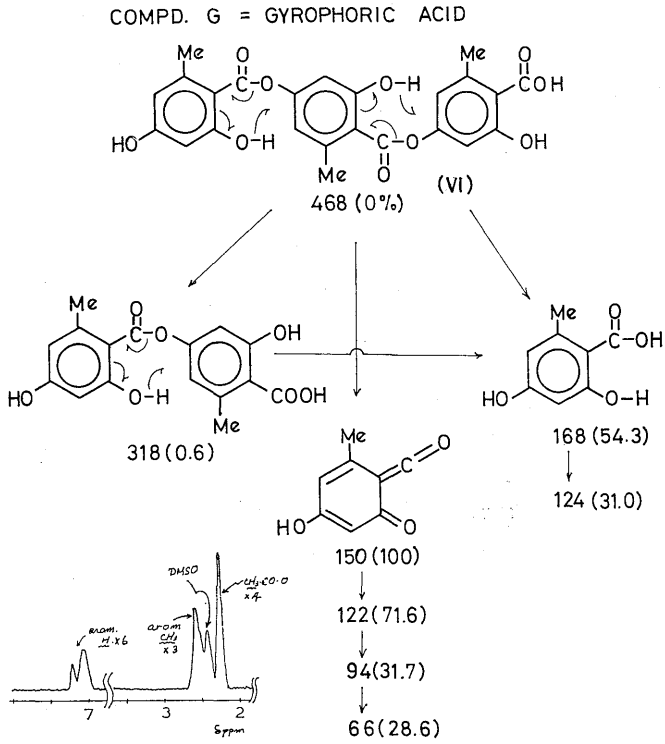
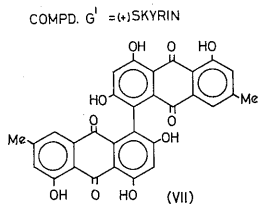


Fig. 7. The fragmentation pattern of mass spectrum of gyrophoric acid.

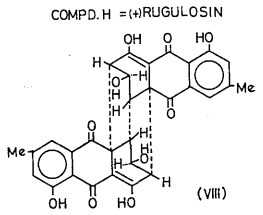
established by the comparison of IR spectra with the authentic sample. The ORD curve of the compound G′ showed (+) Cotton effect at the highest wavelength region.



Compound H (=(+) Rugulosin (VIII))[6,8,9,10], yellow crystals, m. p. (not determined), yield: 10 mg from 65 g of thalli—The Rf-value of the thin layer chromatogram, the colour reaction and the ORD curve suggested that the compound H would be (+)-rugulosin. The identity was proved by the comparison of IR spectra with the authentic sample of (+) rugulosin obtained from *Penicillium brunneum* Udagawa.

(+) Skyrin and (+) rugulosin are occurring together as the metabolites

of various fungi, such as *Penicillium rugulosum* Thom., *P. tardum* Thom., *P. variabile* Sopp., *P. brunneum* Udagawa, *Endothia parasitica* (Murr.) P. J. et H. W. And., *E. fluens* (Sow) Shear. et Stevens. (+) Skyrin was also found being accompanied by other colouring matters in the metabolites of *Penicillium islandicum* Sopp.



The structure of rugulosin has recently been formulated as (VIII). It would be noted that *Acroscyphus sphaerophoroides* produces such fungal colouring matters, skyrin and rugulosin, besides the common lichen metabolites, usnic acid, atranorin, gyrophoric acid, and zeorin. The formation of calycin in this lichen is also noted as this compound has only been found so far in some species of *Lepraria, Sticta* and *Candellariella.*

Acknowledgements The authors wish to thank Prof. Emeritus Y. Asahina and Dr. S. Kurokawa, National Science Museum, Tokyo, for their kind advices in lichenology. Thanks are also due to Ministry of Education for grant supporting the Botanical Expedition to Eastern Himalaya organized by the University of Tokyo.

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本地衣は我国に於ては極めて珍らしいが，環太平洋地域の高山で時々発見される。今回はヒマラヤ地方ブータン国内で採集したものを抽出し calycin, chrysophanol, atranorin, usnic acid, zeorin, gyrophoric acid, skyrin および rugulosin を得た skyrin, rugulosin は *Penicillium rugulosum* その他の糸状菌の代謝産物として得られている色素でこれが地衣成分として見出されたことは興味深い。